observation period was completed (i.e., that is, the 58th day after the first administration). The main hematological test indicators included white blood cells (WBC), red blood cells (RBC), platelets (PLT), neutrophils (Neuts) and lymphocytes (Lymph), etc. 100 μL of whole blood was collected from each test animal. Due to the limitation of volume, these samples were diluted 3 times to achieve the final blood cell counts. On basis of the quantitative analysis of the modified dilution factor, the results were expressed as mean ±SD.

[0637] After receiving the treatment of the ADC containing ring-opening methyl maleate linker (W') of the present application, the animals of three dose groups (1 mg/kg, 2.5 mg/kg, 5 mg/kg) were subjected to hematological analysis and the results were shown in FIG. **11**, in which the numbers "1" and "2" represented the results of whole blood analysis of test animal on the 28th and 58th days after administration, respectively. The ANOVA analysis results of the hematological parameters of the two batches of blood samples showed that there was no significant difference between the solvent control group and the test compound treatment groups (1 mg/kg, 2.5 mg/kg, and 5 mg/kg). The above conclusions indicated that the ADC containing ring-opening methyl maleate linker provided in the present application had low blood toxicity and off-target bone marrow toxicity, which could also be the main reason why it was well tolerated.

EXAMPLE 19

Histopathological Analysis of MAB-W'-C_6-Val-Ala-PAB-MMAE at Therapeutic Dose in Xenograft Model

[0638] When the test in BT-474 xenograft model was completed, the inventors performed histopathological study on the animals treated with the ADC (MAB -W'-C_6-Val-Ala-PAB -MMAE) containing ring-opening methyl ester linker (W'). The test organs mainly included bone marrow, heart, liver and lung. Tissue samples of heart, liver, and lung were fixed in 10% formalin for 24 hours and transferred to 70% ethanol, dehydrated with 70% ethanol, 85% ethanol, 90% ethanol, 95% ethanol, 100% ethanol, and treated with xylene for 3 times, then embedded with paraffin, sectioned and subjected to H&E staining. The bone marrow was made into a smear. The tissue sections and bone marrow smear were observed with magnifying glass, and pictures were taken under microscope. The results were shown in FIG. **12**. Each administration group contained 6 animals, and the original size of picture was 425×320

[0639] The results showed that compared with the solvent control group, the test compound treatment groups also showed no significant difference. This experiment further confirmed the safety of using the ADC provided in the present application.

EXAMPLE 20

Large-Dose Tolerance Test of MAB-W'-C_6-Val-Ala-PAB-MMAE (DAR: About 4)

[0640] In this example, on a normal CD-1 mouse model, the maximal tolerance dose of the ADC (MAB-W'-C_6-Val-Ala-PAB-MMAE, DAR was about 4) containing ring-opening methyl ester linker (W') was evaluated.

[0641] The CD-1 mice [Crl:CD-1 (ICR)] used in the experiment were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd., about 7 to 9 weeks old, about 22 to 40 g for males, and 20 to 35 g for females. Each test drug dose group had 6 animals (half male and half female); the animals were separately fed in mouse boxes with polycarbonate solid bottom and with corn cobs as bedding. The animals freely ate rodent feed and freely drank water in bottle. The environment was controlled to maintain temperature at 20° C. to 26° C. and relative humidity at 40% to 70%, and the lighting of animal room was maintained at light/dark alternation per 12 hours.

[0642] The test drug was dissolved in physiological saline and then injected through tail vein. Five dose groups were set for administration: 10 mg/kg, 20 mg/kg, 40 mg/kg, 80 mg/kg and 120 mg/kg. The volume for administration was 5 mL/kg. The observation period of animals after administration was 15 days, the animals at termination of experiment or the dying animals during experiment were euthanized by inhalation of 70% CO_2/30% O_2. During the observation period after administration, cage-side observation (including the death or dying of animals, the general health condition and symptoms of drug toxicity of animals) was performed twice a day; one day before the administration and 14 consecutive days thereafter, the test animals were subjected to body weight test and detailed clinical observation once per day (including changes in skin, coat, eyes and mucous membranes of animals, as well as changes in respiratory system, circulatory system, autonomic and central nervous systems, physical movement and behavior patterns, etc.).

[0643] In the aforementioned tolerance study of the test drug, after the animals of the dose groups of 10 mg/kg, 20 mg/kg, 40 mg/kg and 80 mg/kg were administrated with the test drug ADC-II (MAB-W'-C_6-Val-Ala-PAB-MMAE), no significant tolerable toxicity and animal death occurred. In the maximum dose group of 120 mg/kg, the body weight of the mice continuously decreased on the 1st to 5th day after the administration, and death occurred in test animals (in 6 test animals 2 died); in addition, all the surviving animals in the test group of 120 mg/kg showed varying degrees of hair loss on the neck, abdomen and limbs from the 9th day after the administration, some test animals had scabs on the hair-loss sites and granular protrusions under skin, and these symptoms were not improved significantly until the termination of the test period (the 15th day after administration). It showed that the dose of 120 mg/kg had reached the maximal tolerance dose (MTD) of ADC-II (MAB-W'-C_6-Val-Ala-PAB-MMAE).

[0644] Taking ADC-II as an example, in the mouse xenograft model test of the HER2-expressing human breast cancer cell line BT-474, when the dose was 2.5 mg/kg, all tumors in the test animals disappeared and did not reoccur within 1 month after the withdraw of drug (Example 16). In the tolerability and safety evaluation study, the dose of 80 mg/kg did not show dose-related toxicity of the test substance, and some animals died when the dose was 120 mg/kg, which indicated that the ADC test compound MAB-W'-C_6-Val-Ala-PAB-MMAE had relatively better tolerability and higher therapeutic index.

[0645] Finally, it should be noted that the above examples are only used to illustrate the technical solutions of the present application and not to limit it; although the present application has been described in detail with reference to the preferred examples, those of ordinary skill in the art should understand that: the specific implementation of the present application can be modified or some technical features can